THE RELATIONSHIP OF P53 GENE MUTATION WITH CLINICOPATHOLOGICAL CHARACTERISTIC IN BREAST CANCER

by

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TABLE OF CONTENTS

I.	Acknowledgements		iii
II.	Abstrak		v
III.	Abstract		vii
1 – INTRODUCTION			1
	1.1	LITERATURE REVIEW	2
	1.2	RATIONALE FOR THE STUDY	9
2 – STUDY PROTOCOL			12
	2.1	DOCUMENT SUBMITTED FOR ETHICAL APPROVAL	13
	2.2	ETHICAL APPROVAL LETTER	36
3 – BODY			
	3.1	TITLE PAGE	44
	3.2	INTRODUCTION	45
	3.3	METHODOLOGY	52
	3.4	RESULTS	57
	3.5	DISCUSSION	60
	3.6	REFERRENCES	63
	3.7	APPENDICES : TABLE AND FIGURES	69

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II. ABSTRAK

Latar Belakang: P53 adalah sejenis genetik penahan tumor. Frekuensi mutasi genetik p53 di dalam kanser payudara adalah 30% (linkungan 15 hingga 71%). Ianya mempunyai kaitan dengan prognosis yang tidak baik. Kaijan ini dijalankan untuk menganalisa mutasi genetik p53 dengan klinikalpathologikal kanser payu dara dan kesesuaian serum pesakit untuk mengesan autoantibodi p53.

Kaedah: Kajian ini dijalankan di Hospital Seberang Jaya dan Institut Perubatan dan Pergigian Termaju, Universiti Sains Malaysia. Seramai enam puluh empat pesakit yang mempunyai tisu segar kanser payudara yang disimpan dibawah -80°C dan data klinikopathologikal yang lengkap dimasukkan didalam kajian ini. DNA dari tisu segar kanser payudara diesktrak dan 10 sampel DNA ini dihantar bagi ujian DNA sekuensi. Sampel selebihnya dijalankan analisa 'Polymerase Chain Reaction'. Serum pesakit-pesakit ini juga diambil untuk mengesan autoantibodi p53.

Keputusan: Purata umur pesakit di dalam kajian ini adalah 52.45±9.51 yrs. Majoriti pesakit adalah berketurunan Melayu iaitu 67.2% diikuti India 17.2 dan Cina 15.6%. Seramai 51.6% pesakit menjalankan CT scan dan 14.1% pesakit kanser sudah di tahap 4 kanser payudara. Kadar mutasi genetik p53 bagi **rs1042522** adalah 15.7% . Bagi **rs59758982,** kadar mutasi adalah 54.7% bagi "Deletion A" dan 45.3% bagi "Wild Type A". **rs35069695** pula menunjukan kadar mutasi "Deletion A" adalah 87.5% dan "Wild Type A" adalah 12.5%. **rs376546152** pula menunjukan kadar mutasi bagi "Deletion GAA" sebanyak 92.2 %. Secara keseluruhan, tiada kaitan antara mutasi genetik p53 dengan perilaku klinikopathologikal kanser payudara kecuali bagi mutasi genetik **rs59758982** menunjukkan kaitan dengan

metastasis di mana p value adalah 0.04. Kajian ini juga menunjukkan bahawa hanya 20.3% pesakit kanser payudara mempunyai sera auto antibodi p53 dan ianya tidak mempunyai kaitan dengan mutasi genetik p53.

Rumusan: Di dalam kajian ini, mutasi genetik p53 didalam tisu segar didapati tiada hubungan dengan klinikopathologikal karakter kanser payudara dan penggunaan serum untuk mengesan autoantibodi p53 adalah tidak konklusif.

Kata Kunci: kanser payudara, mutasi genetik p53, serum autoantibodi p53 serum, rs1042522, rs59758982, rs35069695, rs376546152

III. ABSTRACT

Background: P53 is a tumour suppresor gene. In breast cancer, p53 gene mutation were noted with frequency of about 30% (range 15 to 71%) and associated with poor prognosis. This study was perform determine p53 mutation association with clinicopathological characteristic in breast cancer and to assess the suitability of patients' serum to detect p53 autoantibody.

Methods: This study conducted in Hospital Seberang Jaya and Institut Perubatan dan Pergigian Termaju, Universiti Sains Malaysia. Six four breast cancer patients with available fresh breast cancer tissue that been kept under -80°C and with complete clinicopathological data involve in this study. These fresh breast tissues DNA extracted and 10sampels sent for DNA sequencing. The remaining 54samples proceeded with Polymerase Chains Reaction analysis based on the result from DNA sequencing. The serum of these patients was also taken for p53 autoantibody study using ELISA method.

Results: The mean age of the patients in this study was 52.45±9.51 years. Most of the patients were Malay with 67.2% followed by Indian and Chinese with 17.2% and 15.6% respectively. About 51.6% of these patients undergone CT scan staging and 14.1% has distant metastases. p53 gene mutation prevalence showed **rs1042522** only has 15.7% mutation. There was 54.7% Deletion A and 45.3% Wild Type A detected in **rs59758982**, 87.5% Deletion A and 12.5% Wild Type A in **rs35069695** and 92.2 % recorded for Deletion GAA in **rs376546152**. There was no significant result between these mutation with breast cancer molecular classification and breast cancer aggressiveness except for **rs59758982** shows significant result with p value 0.04 ($p < 0.05$). In regards on for p53 serum auto antibodies,

20.3% of the patients noted to be positive but it has no significant association with p53 gene mutations..

Conclusion: In this study, tissue p53 genetic mutation has no significant association with clinicopathological characteristic of breast cancer and the use of serum p53 auto antibody as biomarkers is inconclusive.

Keywords: breast cancer, p53 gene mutation, p53 serum auto antibodies, rs1042522, rs59758982, rs35069695, rs376546152

1.0 INTRODUCTION

Breast cancer is a distressful disease affecting patient overall wellbeing. It affects both genders and all ethnicity around the world. Each year, this illness affects more than 1 million people worldwide especially women. Worldwide about 1.67million new cases were diagnosed in the year 2012. This represents 12% off all new cases and 25% of all cancer in women.[1] However; the incidence of breast cancers is much lower in men which account for only 1% of all cancer in men.[2]

According to GLOBOCAN 2012, breast cancer is on the rise in developing countries (883000 new cases) compare to the developed countries (794000).[1]

In 2012, it is estimated that worldwide 522000 women succumbed to breast cancer.[1] It is the most frequent cause of cancer death in women in less developed regions and now the second cause of cancer death in more developed area in the world.[1]

National Cancer Registry Malaysia (2007) has reported that, breast cancer was the most frequently diagnosed cancer in women in all ethnic groups with a total of 3,242 cases, accounting for 18.1% of all cancers cases and 32.1% of female cancers.[3,4], Chinese had the highest incidence with an ASR of 46.8 per 100000 population followed by Indian women with an ASR 38.1 per 100000 population and Malay women with an ASR 30.4 per 100000 population. The incidence is steadily increasing with age and peak in the 50-59 age groups.[3,4]

According to Penang Cancer Registry report in 2004 until 2008, there were 1699 cases of breast cancer reported from both government and private hospital.[5] Breast cancer was also the commonest cancer notified among women in Hospital Seberang Jaya with 114 cases in 2012 and 103 cases in 2013.[6,7]

1.1 LITERATURE REVIEW

1.1.1 BREAST CANCER

The pathophysiology of breast cancer has not been fully understood. It is believed to occur as a result of multiple factors involving oncogenes, tumour suppressor gene defect, hereditary gene, reproductive factors, radiation exposure and life-style. Genes whose alterations cause gain-of-function effects are referred to as oncogenes. Meanwhile, genes that cause loss-of-function effects and contribute to the malignant phenotype are known as tumour suppresor genes.[8] So far the known predisposing factors for breast cancer are BRCA1 and BRCA2 genetic mutation, women with first degree relatives with breast cancer at young age, reproductive factors i.e women with prolonged oestrogen exposure (early menstruation, oral contraceptive pills, nulliparous), body mass index more 25 and alcohol consumption more than 10g/day.[4]

Commonly, breast cancer arises either from ductal or lobular cells of the breast tissue. However, there are uncommon type of breast cancer including sarcomas, mucinous, myoepitheliomas and lymphomas.[9]

Currently, breast cancer prognosis is determine by its tumour histological grading, stage based on TNM staging and oestrogen, progesterone HER-2 status.[4] In contrarily, there are many other study has been done on breast cancer including the studies on ki-67, VEGF, p21, CEA, ca 125 and p53 gene to improve breast cancer prognosis and aid in breast cancer management. However, despite all the research that has been done, there is no conclusive result in this matter.[8.9]

1.1.2 P53 AS TUMOR SUPPRESOR GENE

The most studied tumour suppressor gene in all cancers is the p53 gene. Initially; p53 was classified as an oncogene because of it closed relationship with the oncogenic DNA virus

SV40 large T protein. However, isolation of tumour cell complementary DNA demonstrated that p53 in tumour contains a point mutation and has tumour suppressor function.[10] P53 is the first tumour suppressor gene and was identified in year 1979.

P53 gene plays important role to inhibit and eliminate the proliferation of abnormal cells. In normal condition, it is in standby mode. It gets activated once there are cellular stresses such as are genotoxic (DNA alterations induced by irradiation, carcinogens, cytotoxic drugs), hypoxia, oncogenes activation and loss of normal cell contact.[11,12]

P53 also has many mechanisms of anticancer function, and plays a role in apoptosis, genomic stability, and inhibition of angiogenesis. It function via several mechanism; i.e. DNA repair protein when DNA sustained damage, holding cell at GI/S regulating point so repair is possible, and initiate apoptosis if repair impossible.[13] This action occurred in response to cellular stress with main aim to prevent accumulation of genetic changes and damaged cell.[14]

P53 gene is located on the short arm of chromosome 17 (17p13.1).[13] As a 'guardian of the genome', inactivation of p53 gene is a result of p53 deletion, p53 mutation or aberrant p53 function.[10] It can either be a rare germ line mutation (as in case of Li-Fraumeni's Syndrome; autosomal familial disorder) or more commonly a somatic mutation.[12] It's mutation are characterised by a high prevalence of missense mutations found primarily in exons 5-8 in DNA binding domain.[13] The spectrum of mutation in breast cancer is similar to other cancer with less G:C to T:A transversions, and more A:T to G:C transitions.[14] P53 gene act on a damaged cell via its p53 protein. Hence any mutation of the gene will produce mutated p53 protein.

1.1.3 DETECTION OF P53 MUTATION

P53 genetic mutation in breast cancer can be analysed via few methods, but the sensitivity and specificity varies greatly. P53 mutation can be detected by using fresh tumour tissue, fresh paraffin embedded tumour tissue or blood.[15] Molecularly, the detection of p53 mutation can either be by using the DNA or the antibodies. Methods of detection can either be via immunohistoschemically (IHC), single strand conformational polymorphism (SSCP), Polymerase Chain Reaction (PCR) and DNA direct sequencing, genomic microarray, ligase chain reaction or yeast reporter functional assay.[15]

According to *Gilbert et. al* and *Andre. M.O. et. al*, most study in regards of p53 mutation use IHC (paraffin embedded tumour tissue) in view of it being a rapid and inexpensive test comparing to other method.[13,15] However, it is subjected to low accuracy, high false positive and high false negative result. The most accurate test with high specificity is by using yeast reporter functional assay in fresh tumour tissue or blood. However, this test is expensive and mainly use for germline detection of Li Fraumeni syndrome in research. This test needs to be combined with PCR to detect genetic mutation.[10, 15]

The test that is easily available and cost effective is PCR and direct sequencing. These tests are more accurate than SSCP and IHC. Its false negative result is only about 2 to 4%. However, it is limited to detect p53 genetic mutation and not to detect mutation of stabilised protein or posttranslational modifications.[10, 15]

1.1.4 P53 AUTOANTIBODIES IN SERUM

After the discovery of p53 as tumour suppressor gene, *Crawford et. al* proceeded with further study in this matter by assessing the presence of autoantibody against p53 in serum of patients with breast cancer. Out of 155 patients with breast cancer, 14(9%) patients were reported to have p53 autoantibody circulating in their serum.[16] From this pioneer study,

multiple subsequent study were done to analysed the presence of p53 autoantibody in serum and its suitability as tumour biomarkers and prognostic indicator in breast cancer.

The presence of P53 autoantibody in breast cancer patient is still in debates. It was postulated that accumulation of p53 in the tumour is the major factor that triggers the development of the humoral immune response.[17] However; several studies showed that this is not the case. Some patient with detectable p53 autoantibody in the serum have undetected level of p53 in the tumour using IHC method.[18, 19] It was also postulated that p53 autoantibody is the result of p53 genetic mutations.[17]

In a study by *G.A Balogh et.al,* out of 55 patients, 16.36% were found positive for p53 autoantibody and 52.73% positive for p53gene by IHC method. However, all positive p53 autoantibody has positive p53 IHC evaluation.[9]

On the other hand a study by *K. Angelopopou et.al*. on 16 patients using PCR and serum antibody found only 5 patients have detectable mutation in p53 gene as well as antibodies. The abnormalities detected were missense point mutations and nonsense mutations.[17]

Another study by *T.I Hewala et.al* showed that the presence of serum p53 autoantibody remain the same pre and post surgery as well as post chemotherapy using FAC regimes (5-Fluorouracil, Adriamycin and Cyclophosphamide). This can either be good as this can be used to detect the p53 autoantibody despite procedures done but bad as a monitoring method of the treatment response.[20]

1.1.5 EPIDEMIOLOGY OF P53 MUTATION IN BREAST CANCER

P53 is deleted or mutated in up to 50% of all cancer.[21] In breast cancer, p53 gene mutation were noted with frequency of about 30% (range 15 to 71%).[22,23]

A study done in Malaysia by *Mdzin R et. al.*, using immunohistochemistry method, p53 over expression was detected in 45.8% of the cases (27 patients out of 59 patients).[24]

In another study done by *FS Al-Joudi et. al.*, 29.6% p53 overexpression detected in 382 breast cancer patients using immunohistochemistrical method.[25]

According to *Daphne SC Lee et. al.,* in young breast cancer patients (less than 35years old), 6% (5/83) of Asian BRCA negative patients has exonic germ line mutation of p53.[26] In association with other germ line mutation, p53 mutation is also found commonly in BRCA1 and BRCA2 patients.[15]

Histologically, frequency of p53 genetic mutation was found highest in medullary breast cancer and lowest seen in papillary or mucinous type.[15] p53 mutations are also more common in ductal carcinoma than in lobular carcinoma.[10,27] p53 abnormalities also associated with higher grade cancers, aneuploid tumours and high mitotic rate tumours.[12,28]

Beside that, p53 mutation in breast cancer is widely reported to be associated with low level of oestrogen and progesterone receptors.[27,28,29,30] Oestrogen receptors(ER) and progesterone receptors(PR) are known markers for less aggressive tumours with good response for hormonal therapies.[28]

Furthermore, *Pavel Rossner J. et.al.* also found that women with ER/PR negative tumours had almost 4 fold higher risk of having p53 mutation comparing to ER/PR positive patient.[31]

1.1.6 P53 IN BREAST CANCER PROGNOSIS AND SURVIVAL

P53 gene mutation is associated with high grade tumour (histology grade III and more), worse tumour staging (stage III and IV), triple negative breast cancer and reduced survival rates. [4,12,23,28] Triple negative breast cancers are defined as lack of oestrogen receptors

(ER), progesterone receptors (PR) and c-erB-2/Her2 expression.[32] These triple negative patients are not responsive to hormonal therapy and monoclonal antibodies treatment.

The known importance of detecting mutant P53 gene in breast cancer tissue is its roles as prognostic indicator. Multiple studies have shown that p53 mutation were associated with worse prognosis and shorter survival rates. However, few studies also indicate that the presence of p53 as independent prognostic indicator is weak.[12] Thus; combining with other indicators such as ER/PR, cerB-2/HER-2 and Ki-67 is more effective as prognostic indicator.

It was found by *Hiroko Yamashita et.al* that coexistence of HER2 over expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer.[33]

In study done by *Tadahiro N et.al,* sera positive p53 autoantibody patients is significantly correlated with breast cancer histological grade 3and above (p value= 0.002). They also found that the proportion of triple negative tumours significantly higher among this patient (p=0.003).[34]

Kulie a et. al. also gave the same result as their study found that sera positive p53 autoantibody in breast cancer patients has higher tumour size, higher tumour grade, positive axillary lymph nodes, and ER/PR negativity.[35]

Another concern in prognosis and survival of breast cancer patients is metastases. According to *P. Yang et. al*., high p53 expression was associated with advanced TNM stage (p=0.011), multiple organ involvement (p=0.066) and shorter disease free survival (p=0.004).[36]

A molecular study by *Antti A et.al*., found that mutant p53 breast cancer tumour initiated myosin-X (Myo-10) dependent cell invasion cascade. Myo-10 is highly expressed in breast cancer and mediates adhesion, migration, invasion and metastases of cancers cell in vitro and in vivo.[37]

Regarding survival in patients with p53 mutations, a study on 700 breast cancer patients showed disease free survival rates at 5years was only 58% for high positive immunochemistry p53 tumours as compare to 80% in negative p53 tumour group.[28]

Pavel Rossner J et.al., found that any p53 mutations and missense mutations were associated with breast cancer specific mortality (Hazard Ratio HR = 1.7, 95% CI = 1.0-2.8) and all cause of mortality (HR=1.5, 95% CI = 1.0-2.4).[14]

1.1.7 P53 IN CHEMOTHERAPY

The poor prognosis of p53 mutation breast cancer patients may be related to the fact that this mutation rendered them resistance to certain chemotherapy drug. Anticancer drugs act by few ways either by inducing cancer cells apoptosis or causing cell instabilities. Example of anticancer drug that is involved in cell apoptosis is the anthracyclines–based drug. In an apoptotic genetic program mutations tumour, it could produce insensitivity towards this group of anticancer drug.[4,39]

A study conducted by Daniela Kandioler-Eckersberger et.al on value of p53 to predict cytotoxic effect of two commonly used chemotherapy regimes in breast cancer showed that, in patient with 5-Fluorouracil, Epirubicin, Cyclophosphamide (FEC) group, treatment failure was related to p53 mutation group. The study also showed that there is significant association between abnormal p53 and respond to Paclitaxel. This is believe due to, FEC regimes considered to induced p53-dependent apoptosis comparing to Paclitaxel that is independent of p53 function.[40]

Several studies showed that tumour carrying p53 gene mutations might be less sensitive to anthracyclines–based drugs (i.e. epirubicin) comparing to Taxanes-based (i.e. Paclitaxel).[41,42] A study by Clahsen et. al. also showed that p53 accumulation in response to anthracycline-containing regime was associated with poor response.[4]

1.2 RATIONAL OF THE STUDY

1.2.1 RESEARCH JUSTIFICATION AND BENEFITS

P53 genetic mutation in breast cancer in Malaysia is not widely studied. This study provided a local data of p53 genetic mutation and its association with clinicopathological characteristic in breast cancer. Furthermore, most studies that have been conducted with regards to p53 mutation in breast cancer in Malaysia were using formalin fixed breast cancer tissue and enzyme immunohistochemistry assay to detect p53 mutation.[13,24,25]

In this study fresh breast cancer tissue that has been preserved in -80°C and allele specific PCR technique was use to detect p53 mutation. P53 allele specific PCR technique is proven more accurate in detecting p53 genetic mutation.[13,15] Concurrently, the same patients' serum was used to detect p53 autoantibody. This was to determine the suitability of p53 autoantibody in serum as biomarkers in breast cancer patient and aid future management in breast cancer patient.

The main focus of current study was to evaluate p53 genetic mutation amongst fresh breast cancer tissue of patient undergone operation and p53 autoantibody in serum. So far, data on p53 genetic mutation in breast cancer especially locally is not widely available. The possibility to use p53 autoantibody in serum as tumour biomarkers is also yet to be discovered and discussed.

1.2.2 RESEARCH QUESTION

1. Is there any significant association between the clinicopathological characteristic with p53 mutation in breast cancer?
2. Can serum p53 autoantibody be used as biomarkers in breast cancer?

1.2.3 RESEARCH LIMITATIONS

These study limitations include small sample size in comparison with incidence of breast cancer in the country. Furthermore, the duration of available to conduct this study was also limited as even though nineteen mutation and deletion was found in this study, only one mutation and three deletion can be perform for all samples.

1.2.4 OBJECTIVES

GENERAL OBJECTIVES

1. To determine p53 mutation association with clinicopathological characteristic in breast cancer
2. To assess accuracy of patients' serum in detecting p53 autoantibody

SPECIFIC OBJECTIVES

a) To determine the profiling data (age, ethnicity, duration of symptoms, risk factor, clinicopathology staging) of women with breast cancer
b) To determine prevalence of p53 mutation in Malaysian's fresh breast cancer tissue using PCR technique
c) To ascertain fresh breast cancer tissue p53 mutation association with ER, PR, HER-2 status in patient with breast cancer
d) To analyse p53 mutation in relations of breast cancer aggressiveness i.e; staging (according to Malaysian Clinical Practise Guidelines) and grading
e) To evaluate for serum p53 autoantibody suitability as biomarkers in breast cancer.

1.2.5 HYPOTHESIS

ALTERNATIVE HYPOTHESIS (HA)

a. There is significant association between clinicopathological characteristic and p53 mutation in breast cancer
b. Serum p53 autoantibody can be use as biomarkers in breast cancer

NULL HYPOTHESIS (HO)

a. There is no significant association between clinicopathological characteristic and p53 mutation in breast cancer
b. Serum p53 autoantibody cannot be use as biomarkers in breast cancer

2.0 STUDY PROTOCOL

2.1 DOCUMENT SUBMITTED FOR ETHICAL APPROVAL

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PARTIAL REQUIREMENT FOR MASTER OF MEDICINE (SURGERY) PROGRAMME

UNIVERSITI SAINS MALAYSIA

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1. INTRODUCTION

1.1. OVERVIEW

Breast cancer is a devastating illness as it affects patient's self-confidence and one of the commonest causes of death due to cancer in woman. Breast cancer is one of the commonest non squamous cell carcinoma diagnosed worldwide accounts about 1.38million in 2008.[1] National Cancer Registry Malaysia (2007) reported that in Malaysia, the female breast cancer is the most frequently diagnosed cancer in women in all ethnic groups with total number of 3,242 cases accounted for 18.1% in all cancers cases reported and 32.1% female cancers cases.[2]

From Penang Cancer Registry report 2004 till 2008, the reported breast cancer within that period was 1699patients from both government and private hospital.[3] Breast cancer is also the commonest cancer notified among women in Hospital Seberang Jaya with 114 cases in 2012 and 103 cases in 2013.[4,5]

Breast cancer believed to occur in result of multiple factors involving oncogenes, tumour suppressor gene defect, genetic, environment and life-style. The most studied tumour suppressor gene in all cancers is the p53 gene. P53 gene plays important role to inhibit and eliminate the proliferation of abnormal cells. In normal condition, it is in standby mode. It gets activated once there are cellular stresses.[6]Examples of the stresses are genotoxic (DNA alterations induced by irradiation, carcinogens, cytotoxic drugs), hypoxia, oncogenes activation and loss of normal cell contact.[7]In breast cancer, p53 gene mutation changes noted with frequency of about 30% (range 15 to 71%).[8,9] P53 mutation is associated with high grade tumour (histology grade III and more), worse tumour staging (stage III and IV) and reduced survival rates. [9,10,11]

Anticancer drugs act on cancer cell either by inducing apoptosis or cause cell instability. According to Malaysian Clinical Practice Guideline on Management of Breast Cancer November 2010, all early breast cancer patients should receive chemotherapy.[12]Anticancer drug that involve in apoptosis example is anthracyclines–based drug. However, a genetic program mutations in apoptotic pathway could produce resistance tumour.[13,14]Few present studies done indicate that tumour carrying p53 gene mutations might be less sensitive to anthracyclines–based drugs (i.e. epirubicin) comparing to Taxanes-based (i.e. Paclitaxel).[15,16]

1.2. LITERATURE REVIEW

Breast cancer affect both gender, all ethnicity around the world. Commonly, breast cancer arises either from ductal or lobular cells of the breast tissue. However, uncommon type of breast cancer included sarcomas, myoepitheliomas and lymphomas.[17] Breast cancer prognosis is currently determine by its tumour histological grading, stage based on TNM classification, estrogen, progesterone and HER-2 status.[2] Beside this, there were many tumour markers and genetic study has been performing in breast cancer. These include the studies onki67, VEGF, p21,CEA, ca 125 and p53 gene.[10,13,15]

P53 is the first tumour suppressor gene identified in year 1979. P53 has many mechanisms of anticancer function, and plays a role in apoptosis, genomic stability, and inhibition of angiogenesis. It function via several mechanism; i.e. DNA repair protein when DNA sustained damage, holding cell at GI/S regulating point so repair is possible, and initiate apoptosis if repair impossible.[18] This action occurred in response to cellular stress with main aim to prevent accumulation of genetic changes and damaged cell.[11]

p53 gene located on the short arm of chromosome 17 (17p13.1).[18] It's mutation are characterised by a high prevalence of missense mutations found primarily in exons 5-8 in DNA binding domain.[11] The spectrum of mutation in breast cancer similar with other cancer with less G:C to T:A transversions, and more A:T to G:C transitions.[11]P53 gene act on a damaged cell via its p53 protein. Hence any mutation of the gene will produce mutant p53 protein.

G.A Balogh et.al study showed that serum mutant p53 protein was elevated in invasive breast carcinomas with strong correlation with accumulation of mutant p53 detected by immunohistochemical.[19]

The known important of detecting mutant P53 in breast cancer tissue is its release prognostic indicator. Multiple studies have shown that p53 mutation associated with worse prognosis and shorter survival rates. According to *P. Yang et.al.*,high p53 expression was associated with advanced TNM stage (p=0.011), multiple organ involvement (p=0.066) and shorter disease free survival (p=0.004).[20] *PavelRossner J. et.al.*found that women with ER/PR negative tumours had almost 4 fold higher risk of having p53 mutation comparing to ER/PR positive patient.[11]

Hiroko Yamashita et.al found that coexistence of HER2 overexpression and p53 protein accumulation is strong prognostic molecular marker in breast cancer.[21]

This poor prognosis may be related to the fact that P53 gene mutation resistance to certain chemotherapy drug. A study conducted by Daniela Kandioler-Eckersberger et.al on value of p53 to predict cytotoxic effect of two commonly used chemotherapy regimes in breast cancer showed that, in patient with FEC group, treatment failure was related to p53 mutation group. The study also showed that there is significant association between abnormal p53 and respond to paclitaxel. This is believe due to, FEC regimes considered to induced p53-dependent apoptosis comparing to paclitaxel that is independent of p53 function.[22]Another study also showed that p53 accumulation in response to anthracycline-containing regime associated with poor response.[23]

1.3. RESEARCH JUSTIFICATION AND BENEFITS

P53 mutation in breast cancer in Malaysia is not widely study. Hence, this study will provide local data of p53 mutation association with clinicopathological characteristic in breast cancer. Besides that, most study has been conducted in regards of p53 mutation in breast cancer is using formalin fixed and immunohistochemical technique in detecting p53mutation in breast cancer tissue. However, in this study fresh breast cancer tissue that has been preserved in control environment is use to detectp53 mutation using p53 alelle specific PCR technique. Concurrently, the same patients' serum tissue to detect p53 autoantibody. Hence, this will determine the suitability of p53mutation in blood or present of p53 autoantibodies in serum as biomarkers in breast cancer patient.

1.4. OBJECTIVES

1.4.1. GENERAL OBJECTIVES

To determine p53 mutation association with clinicopathological characteristic in breast cancer

1.4.2. SPECIFIC OBJECTIVES

a. To determine the profiling data (age, ethnicity, duration of symptoms, risk factor,clinicopathological) of women with breast cancer
b. To determine **prevalence of p53 mutation in Malaysian's fresh breast cancer tissue using PCR technique**

c. To ascertain **fresh breast cancer tissue p53 mutation association with ER, PR, HER-2 status in patient with breast cancer**
d. To analyse p53 mutation in relations of breast cancer aggressiveness i.e; staging (according to Malaysian Clinical Practise Guidelines) and grading
e. To **look for serum p53 autoantibody suitability as biomarkers in breast cancer.**

1.5. RESEARCH QUESTION

1. Is there any significant association between the clinicopathological characteristic with p53 mutation in breast cancer?
2. **Can serum p53 autoantibody be used as biomarkers in breast cancer?**

1.6. HYPOTHESIS

1.6.1. ALTERNATIVE HYPOTHESIS (HA)

a. There is significant association between clinicopathological characteristic and p53 mutation in breast cancer
b. Serum p53 autoantibody can be use as biomarkers in breast cancer

1.6.2. NULL HYPOTHESIS (HO)

a. There is no significant association between clinicopathological characteristic and p53 mutation in breast cancer
b. Serum p53 autoantibody cannot be use as biomarkers in breast cancer

2. METHODOLOGY

2.1. RESEARCH DESIGN

This is a Cross-Sectional study

2.2. STUDY VENUE

This study will be conducted in Hospital Seberang Jaya and Institut Perubatan dan Pergigian Termaju (IPPT), USM

2.3. STUDY DURATION

This study will be conducted from 1st January 2015 to 30th June 2016

2.4. STUDY POPULATION

Female patients diagnosed with breast cancer in year 2012 until 2015 from Hospital Seberang Jaya that undergone mastectomy with axillary sampling or clearance or wide-local excision with axillary sampling or clearance.

2.5. SAMPLE SIZE

Sample Size Calculation:

Sample size was calculated using sample size calculator for prevalence studies, Naing et. al, 2006)

Estimated prevalence of p53 mutation= 23.6 % (Anita Langerodet. Al; TP53 mutation status and gene expression profiles are powerful prognostic markers of breast cancer, May 2007)[24]
Study precision = 11%
n= 58

Anticipating a 10% attrition rate, therefore, the expected no of sample is 64.

2.6. SELECTION CRITERIA

2.6.1. INCLUSION CRITERIA

a. Patient with histologically confirmed of breast cancer.
b. Breast cancer's patient that undergone Mastectomy with Axillary Sampling or Axillary Clearance or Wide-Local Excision with Axillary Sampling or Axillary Clearance
c. Cases with completed histopathological report including grading, staging, ER, PR and HER-2 status
d. Patient with clinical staging done. (Stage I and II did ultrasonography of the abdomen and chest x-ray, Stage III and IV did CT scan of thorax, abdomen, pelvic as Malaysian Clinical Guidelines of Breast Cancer)
e. Patient consented for the p53 mutation study and serum mutant p53 auto antibodies study
f. Patient more than 18 yea

2.6.2. EXCLUSION CRITERIA

a. Patient do not consented for p53 mutation study and serum p53 autoantibody study
b. Patient who has passed away prior to the time this study conducted
c. Incomplete documentation of patient's case note

2.7. CONSENT FOR STUDY

Each patient will be explained regarding the objective and method of the study by primary investigator. Informed consent is taken in accordance to Declaration of Helsinki, with protocol and statement of informed consent approved by Ethnics Committee. Confidentiality of the patient is keep by giving **Code Number** to patient. Only the primary investigator has the list of patient identification card number or hospital registration number for reference.

Patient has no access onto his / her own personal information and study data. However, if patient wish to know their result, they are free to call primary investigator to get that information.

2.8. RESEARCH TOOLS AND DATA COLLECTION

Data collection sheet (proforma) is design to obtain the information from patient's case record. Patient is labelled using **Code Number** to protect patient privacy. Patient personal data will only be known by primary investigator where it will keep in a list stating patient identification number or registration number. The details on demographic including age at diagnosis and ethnicity will be abstracted. The data on mode and symptoms at presentation, family history, clinical characteristic, histopathological findings, and chest x-ray and ultrasound abdomen or CT scan thorax abdomen pelvic will be obtained from patient's medical record. Patient serum is collected on diagnosis.

Serum mutant p53 protein from 10 normal female individual (without any breast disease and family history of breast cancer) taken as negative control to test the p53 ELISA^{PLUS} (auto antibody) kit (Oncogene Research Product, Cambridge MA, USA).

2.9. TISSUE P53 AND SERUM P53 TESTING

Tissue:

Patients' breast cancer tissue will be collected after surgery without being fixed by formalin. It will be collected the same day of operation by IPPT staff. The primary breast cancer tissue was will be snap frozen and stored at -80^{o}C. Frozen section will be stained with hematoxylin/eosin and were will be reviewed to confirm tumour content by the pathologist.

Allele-specific PCR:

DNA will be extracted from patients' tissue and blood samples using Blood/Tissue Midi Kit (Qiagen). The presence of mutation in p53 genes will be detected by allele-specific PCR.

Serological analysis:

3ml blood from patient taken at diagnosis in plain bottle. It the centrifuge to obtain the serum and separate it from the blood.

The present of p53 autoantibodies from serum of all patients will be detected using the p53 ELISA^{PLUS} (auto antibody) kit (Oncogene Research Product, Cambridge MA, USA). The kit will be used to measure circulating antibodies to p53 in human serum samples.

Serums from 10 normal healthy women with no history of breast and any cancers will be taken as negative control.

2.10. STATISTICAL ANALYSIS

The data will be analysed using Statistical Package for the Social Sciences (SPSS) software version 20. Independent T, Pearson's Chi Square and Fisher's Exact Test will be used to determine the association. *P* value of less than 0.05 is considered statistically significant

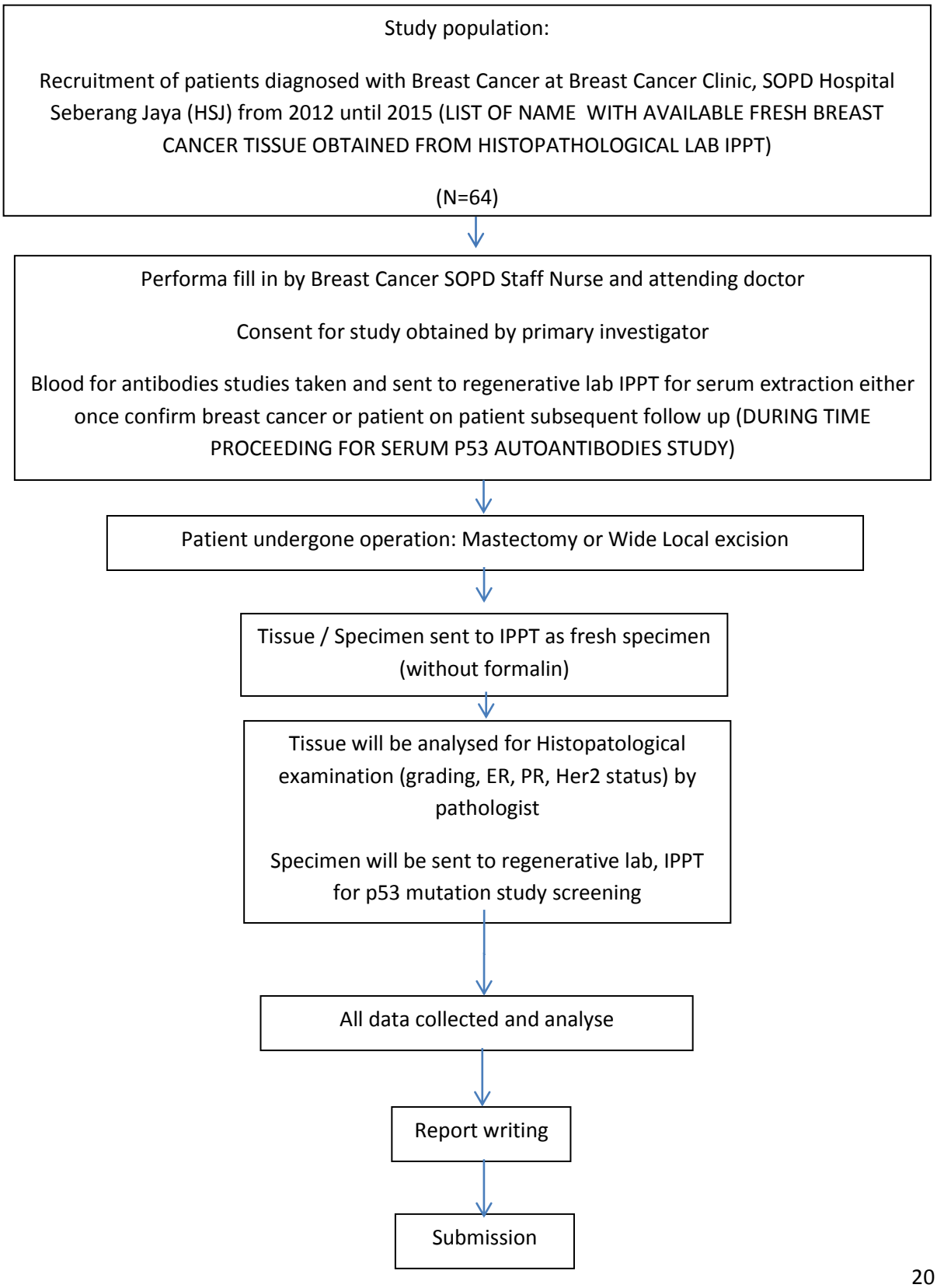
2.11. ETHICAL APPROVAL

Ethical approval applied before conducting the study as patient's tissue and body fluid is needed in this study from UniversitiSains Malaysia Ethical Committee and KementerianKesihatan Malaysia.

2.12. STUDY GRANTT

This study is approved under short term grant Institut Perubatan dan Pergigian Termaju by Division of Research and Innovation, University Sains Malaysia.

3. FLOW CHART



4. BUDGET

Grantt Approval from RCMO, USM with grantt No, CIPPT/6313162

5. GANTT CHART

6. PLANNED MILESTONE

a. JUNE 2015 :COMPLETION OF DATA COLLECTION
COMPLETION OF DATA ANALYSIS
b. DECEMBER 2015 : PRESENTATION AND REPORT SUBMISSION
c. MARCH 2015 : WRITING
d. JUNE 2015 : SUBMISSION

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BORANG ETIKA - 02

BORANG MAKLUMAT DAN KEIZINAN PESAKIT/ SUBJEK
PATIENT INFORMATION AND CONSENT FORM
(PROJEK PENYELIDIKAN)

MAKLUMAT KAJIAN

Tajuk Kajian: Kaitan Mutasi Genetik p53 dengan Perilaku Klinikal-pathologi Kanser Payudara

NamaPenyelidik: DR. FITREENA ANIS AMRAN

No. Pendaftaran MMC : 49486

PENGENALAN

Anda dipelawa untuk menyertai satu kajian penyelidikan secara sukarela untuk kajian mutasi genetic p53 didalam pesakit kanser payudara. Genetic p53 merupakan satu gene yang penting dalam pengawal sel-sel tumbuh. Apabila genetik ini mengalami mutasi, sel-sel tubuh tidak lagi terkawal maka terjadilah tumor. Tumor yang agresif akan menjadi kanser. Kliniko-pathologikal bermaksud, keadaan pesakit semasa pemeriksaan dari segi saiz ketumbuha, perebakan kanser ke bahagian lain dan kajian keatas tisu specimen pesakit. Serum p53 autoantibodi pula adalah di mana apabila p53 mutasi berlaku ianya akan menghasilkan protein yang bermutasi. Untuk memusnahkan protein ini tubuh manusia akan menghasilkan antibody secara semula jadi kepada protein yang bermutasi tersebut. Kajian ini melibatkan pengambilan darah dari pesakit dan pengambilan tisu kanser payudara dari specimen pembedahan.Satu salinan borang maklumat ini akan diberikan kepada anda sekiranya anda bersetuju menyertai kajian ini.

TUJUAN KAJIAN
Kajian ini adalah untuk melihat samaada mutasi genetic p53 berkaitan dengan perilaku klinikal-patologi kanser payudara.

KELAYAKAN PENYERTAAN

Doktor yang bertanggung jawab dalam kajian ini atau salah seorang kakitangan kajian telah membincangkan kelayakan untuk menyertai kajian ini dengan anda. Adalah penting anda berterus terang dengan doktor dan kakitangan tersebut tentang sejarah kesihatan anda. Andatidakseharusnya menyertai kajian ini sekiranyaandatidakmemenuhisemuasyaratkelayakan.

1. **Ada telah dikenalpasti menghidap penyakit kanser payudara**
2. **Anda telah menjalani pembedahan kanser payudara**
3. **Laporan patologi anda lengkap termasuk 'grading, 'staging',ER,PR dan HER-2' status**
4. **Berumur 18 tahun ke atas**

PROSEDUR-PROSEDUR KAJIAN

Pada lawatansusulan anda, setelah anda dikenal pasti menghidapi kanser payudara dari sampel tisu biopsi pada lawatan pertama anda akan diterangkan mengenai kajian ini. Sekiranya anda setuju menyertai kajian, darah anda sebanyak 3ml akan diambil dan dihantar ke makmal. Anda akan seterusnya menjalani pembedahan atau rawatan sebelum pembedahan seperti yang dicadangkan oleh pakar bedah bertugas. Tisu kanser dari pembedahan yang telah dilakukan diambil untuk kajian. Kajian yang dilakukan adalah mengenalpasti mutasi genetik p53 dalam tisu kanser payudara anda dan di dalam darah anda. Selain itu, serum dari darah akan dijalankan ujian autoantibodi p53.